PATENT
Docket No.: **20011/1371**

#13

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Beck et al.	Examiner: B. Coleman
Serial No.	:	09/704,306	
Cnfrm. No.	:	4919	Art Unit: 1624
Filed	:	November 2, 2000	
For	:	NOVEL 4-PHENYL SUBSTITUTED TETRAHYDROISOQUINOLINES AND USE THEREOF	

DECLARATION OF BRUCE F. MOLINO UNDER 35 U.S.C. § 1.132

I, BRUCE F. MOLINO, pursuant to 37 C.F.R. § 1.132, declare:

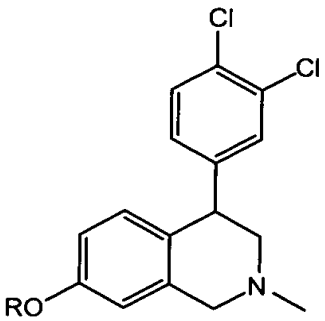
1. I received a B.S. degree in Chemistry from Rutgers University in 1977 and Ph.D, degree in Organic Chemistry from the University of Maryland in 1984.

2. I am the Senior Director of the Medicinal Chemistry Department at Albany Molecular Research, Inc.

3. It is my understanding that Albany Molecular Research, Inc. is the assignee of the above patent application.

4. I present this declaration to demonstrate that compounds of the present application achieve a binding affinity for dopamine transporter ("DAT") to binding affinity for neuroepinephrine transporter ("NET") ratio of at least 2:1 and a binding affinity for serotonin transporter ("SERT") to binding affinity for NET ratio of at least 20:1, while dichlofensine and a metabolite of dichlofensine do not.

5. In particular, in addition to the compounds of the present invention (identified below as the PH-7032 compounds), the following compounds were tested:

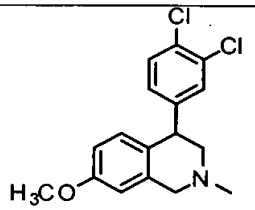
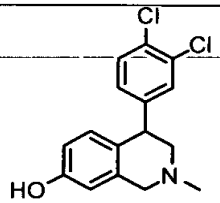
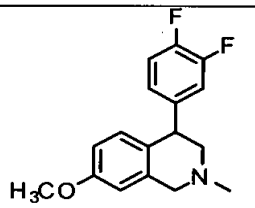
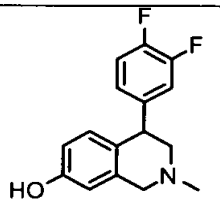
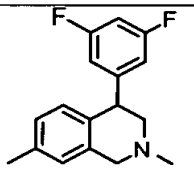


R=Me, dichlofensine, **1**
R=H, metabolite of dichlofensine, **2**

6. In order to evaluate the relative affinity of the various compounds for NET, DAT, and SERT, HEK293E cell lines were developed to express each of the three human transporters. cDNAs containing the complete coding regions of each transporter were amplified by polymerase chain reaction from human brain libraries. The cDNAs contained in pCRII vectors were sequenced to verify their identity and then subcloned into an Epstein-Barr virus based expression plasmid (E Shen, et al., Gene 156:235-239 (1995). This plasmid containing the coding sequence for one of the human transporters was transfected into HEK293E cells. Successful transfection was verified by the ability of known reuptake blockers to inhibit the uptake of tritiated norepinephrine, dopamine, or serotonin.

7. To test compounds for binding, cells were homogenized, centrifuged, and then resuspended in incubation buffer (50mM Tris, 120 mM NaCl, 5mM KCl, pH 7.4). The appropriate radioligand was then added--i.e. [^{3}H] Nisoxetine (86.0 Ci/mmol, NEN/DuPont) was added to a final concentration of approximately 5 nM (for NET binding), [^{3}H] WIN 35,428 (84.5 Ci/mmol) at 15 nM was added (for DAT binding), and [^{3}H] Citolapram (85.0 Ci/mmol) at 1 nM was added (for SERT binding). Various concentrations (10^{-5} to 10^{-11} M) of the compound of interest were then added to displace the radioligand. Incubation was carried out at room temperature for 1 hour in a 96 well plate. Following incubation, the plates were placed on a harvester and washed quickly 4 times with (50 mM tris, 0.9% NaCl, pH 7.4) where the cell membranes containing the bound radioactive label were trapped on Whatman GF/B filters. Scintillation cocktail was added to the filters which were then counted in a Packard TopCount. Binding affinities of the compounds of interest were determined by non-linear curve regression using GraphPad Prism 2.01 software. Non-specific binding was determined by displacement with 10 micromolar mazindol. The results of these binding assays for the various compounds tested is set forth in Tables 1 and 2 below.

Table 1

Compounds	NET, Ki nM	DAT, Ki nM	Selectivity DAT/NET
 **1** **racemate**	55	24	0.44
 **2** **racemate**	8	14	1.8
 PH-7032 compound Racemate	14	90	6.4
 PH-7032 compound Racemate	30	335	11.2
 PH-7032 compound Racemate	32	302	9.4

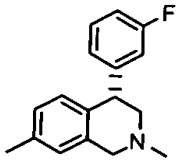
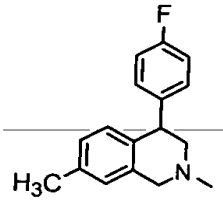
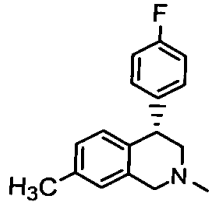
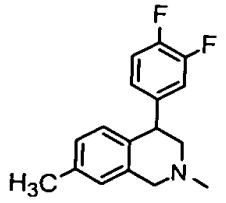
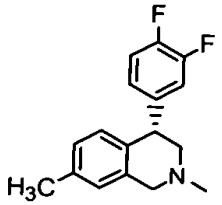
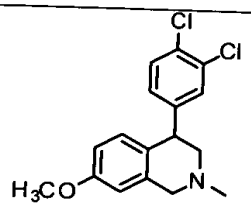
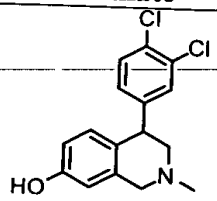
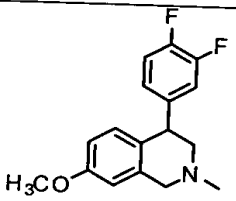
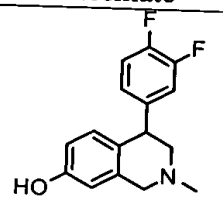
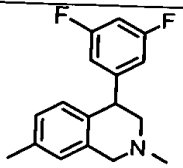
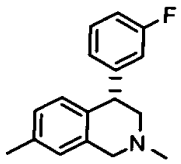
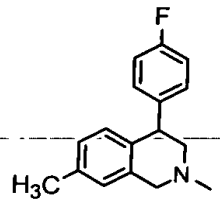
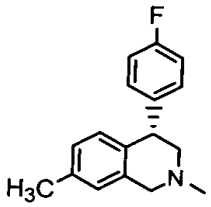
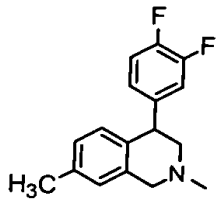
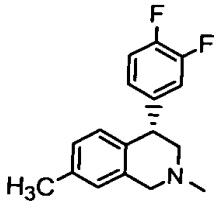
Compounds	NET, Ki nM	DAT, Ki nM	Selectivity DAT/NET
 PH-7032 compound (S)-(+)-enantiomer	12	94	7.8
 PH-7032 compound Racemate	15	76.5	5.1
 PH-7032 compound (S)-(+)-enantiomer	7	36	5.1
 PH-7032 compound Racemate	25.5	180	7.0
 PH-7032 compound (S)-(+)-enantiomer	12.5	136	10.9

Table 2

Compounds	NET, Ki nM	SERT, Ki nM	Selectivity SERT/NET
 **1 racemate**	55	285	5.2
 **2 racemate**	8	13	1.6
 PH-7032 compound Racemate	14	404	29
 PH-7032 compound Racemate	30	867	29
 PH-7032 compound Racemate	32	4597	144

Compounds	NET, Ki nM	SERT, Ki nM	Selectivity SERT/NET
 PH-7032 compound (S)-(+)-enantiomer	12	830	69
 PH-7032 compound Racemate	15	396	26.4
 PH-7032 compound (S)-(+)-enantiomer	7	231	33
 PH-7032 compound Racemate	25.5	1607	63
 PH-7032 compound (S)-(+)-enantiomer	12.5	459	36.7

8. The above results demonstrate that compounds **1** and **2** are more selective for DAT (DAT/NET ratio < 1) in the case of dichlofensine (**1**) and non-selective for NET, DAT, or SERT in the case of the metabolite of dichlofensine (**2**). In contrast, the above data shows that the PH-7032 compounds have a DAT/NET selectivity of ≥ 5.1 and a SERT/NET selectivity of ≥ 26.4. Thus, the compounds of the present invention are selective for NET relative to DAT and selective for NET relative to SERT.

9. Compounds that are selective for NET over transporters for other neurochemicals (e.g., DAT and SERT) have more beneficial therapeutic index than other compounds. By being more selective for NET over DAT and SERT (i.e. having a DAT/NET ratio of at least about 2:1 and a SERT/NET ratio of at least about 20:1), as shown above in Tables 1 and 2, the compounds of the present application possess fewer side effects than compounds **1** and **2**.

10. In the treatment of ADHD, drugs like methylphenidate (Ritalin®) possess a high selectivity for the dopamine transporter (DAT) over norepinephrine transporter (NET). The DAT/NET selectivity ratio is 0.1. Methylphenidate is a schedule II controlled substance, because it possesses the potential for addiction, which is also true for other drugs that affect the dopamine transporter (e.g., cocaine). Compounds in this patent, which are more selective for NET rather than DAT, do not affect dopamine levels to the extent that methylphenidate does and should be much less likely to have this liability.

11. Known drugs that are selective Serotonin Reuptake Inhibitors ("SSRI")(e.g., Prozac™) are effective agents for the treatment of depression. No drugs in this class are currently prescribed for ADHD. This class of compounds is associated with certain side effects (e.g., sexual dysfunction) which is attributable to SERT selective nature of these compounds relative to NET. Compounds with the above DAT/NET and SERT/NET ratios do not possess the side effects of the SSRI class of drugs.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: November 4, 2002

Bruce F. Molino

Bruce F. Molino